

STANDARDIZATION OF HOMOGENEOUS INOCULUM OF *AUREOBASIDIUM* ATCC 20524

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Introduction. *Aureobasidium*, is one of the several genera of "black yeasts", characterized by mostly slow-growing, black and pasty colonies. This fungus, under specific conditions and in agar medium (1,2), may present yeast phase and true hyphae. However this specie presents both phases in liquid medium, what difficults to quantify directly the number of microorganisms to be inoculated in order to be constant in all the assays.

The aim of the present work is to define culture conditions to obtain a homogeneous inoculum where one of the phases prevails.

Methodology. *Aureobasidium* ATCC 20524 was grown in plates of basal medium (3) with the following composition (g/L): sucrose, 10; yeast extract, 2; agar, 18 at 30°C during 24 hs. The kinetics of growth was determined by measuring optical density at 620nm, and correlating these data with dry matter in g/L.

Results and Discussion.

As a result of this work, the following methodology arose: Three platinum loops were taken from colonies grown during 24 hs at 30°C in agar medium plates, and transfer to erlenmeyer flasks with 100ml of liquid medium, maintained at 30°C at 170 rpm of agitation during 24 hs. 2 ml of this culture, were subcultured to another erlenmeyer with 100ml of fresh culture medium and cultivated in the same conditions for 24 hs. This strict protocol of culturing ensures the presence of yeast phase mainly.

In order to evaluate if by modifying the culture medium, the morphology remains constant when the inoculum is prepared as is proposed, four assays were performed with the following treatments:

(E1) basal medium, (E2) basal medium + K₂HPO₄ 5 g/L, (E3) basal medium + MgSO₄·7H₂O 0,5g/L, (E4) basal medium supplemented with K₂HPO₄ 5 g/L and MgSO₄·7H₂O 0,5g/L.

The results of this work are shown in the following table:

	E1	E2	E3	E4
	g/L	g/L	g/L	g/L
1	2,13	0,21	0,33	2,05
2	2,28	0,17	0,34	1,90
3	2,20	0,19	0,32	2,02
4	2,18	0,19	0,35	1,95
5	2,13	0,17	0,33	2,05
6	2,12	0,20	0,37	1,91
7	2,22	-	-	1,99
8	2,14	-	-	2,00
Average	2,2	0,2	0,3	2,0
Variance	0,0031	0,0003	0,0003	0,0034

When the methodology proposed of subculturing was applied, the homogeneous yeast phase was got, although the values of biomass obtained were different. It is outstanding that dry matter values are much higher for the basal medium without supplements of salts, which would indicate that the yeast extract supplies the necessary cations and anions in this inoculum production.

Conclusions.

The system of inoculum preparation proposed, is good to achieve a homogeneous yeast phase grown from *Aureobasidium* ATCC 20524 used in our experiments

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Table 1. Dry matter in g/L for the different culture media.